3.3 其他相關技術 Other related techniques

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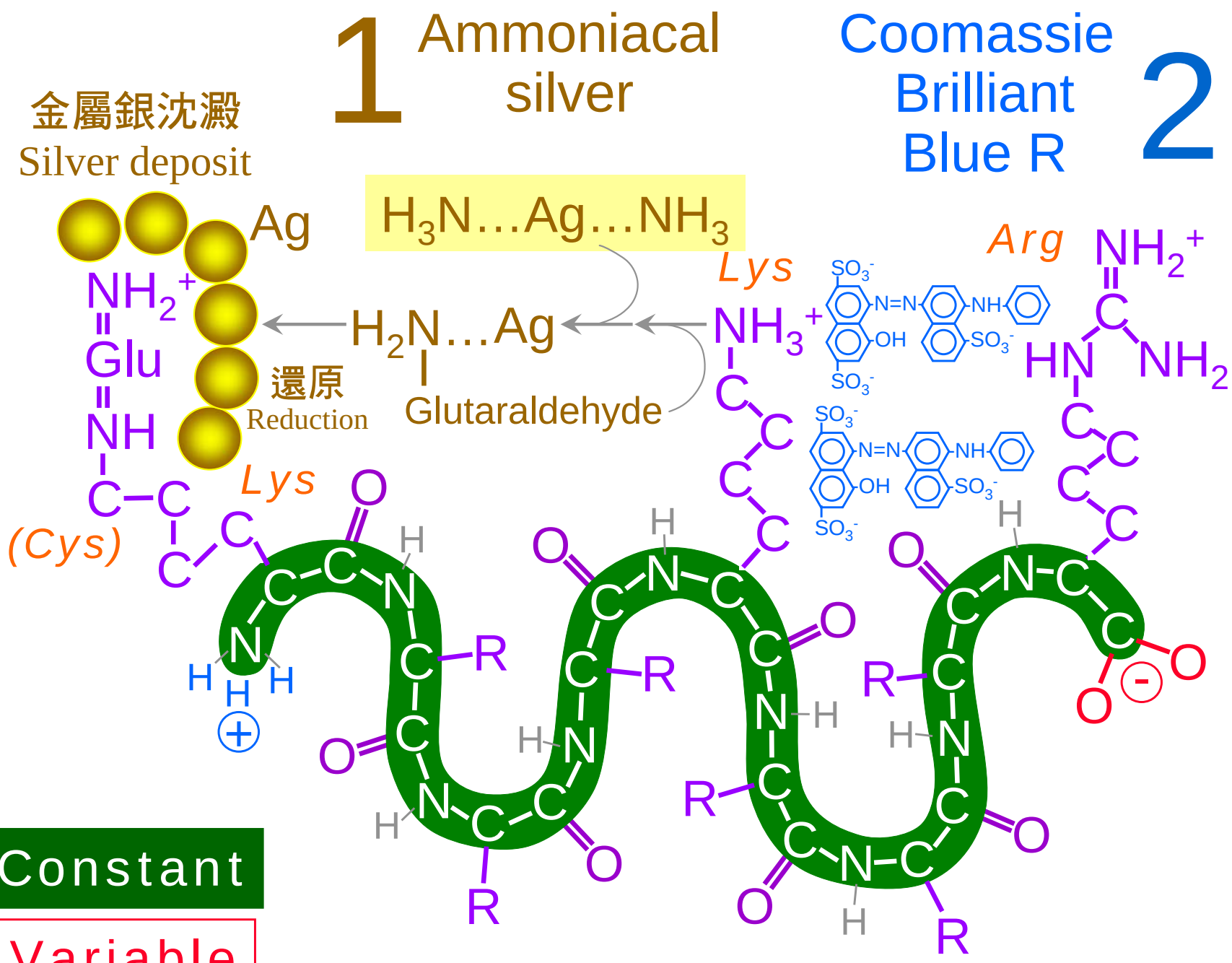
  電泳後可再把蛋白質色帶轉印到薄膜上

各種蛋白質染色法機制

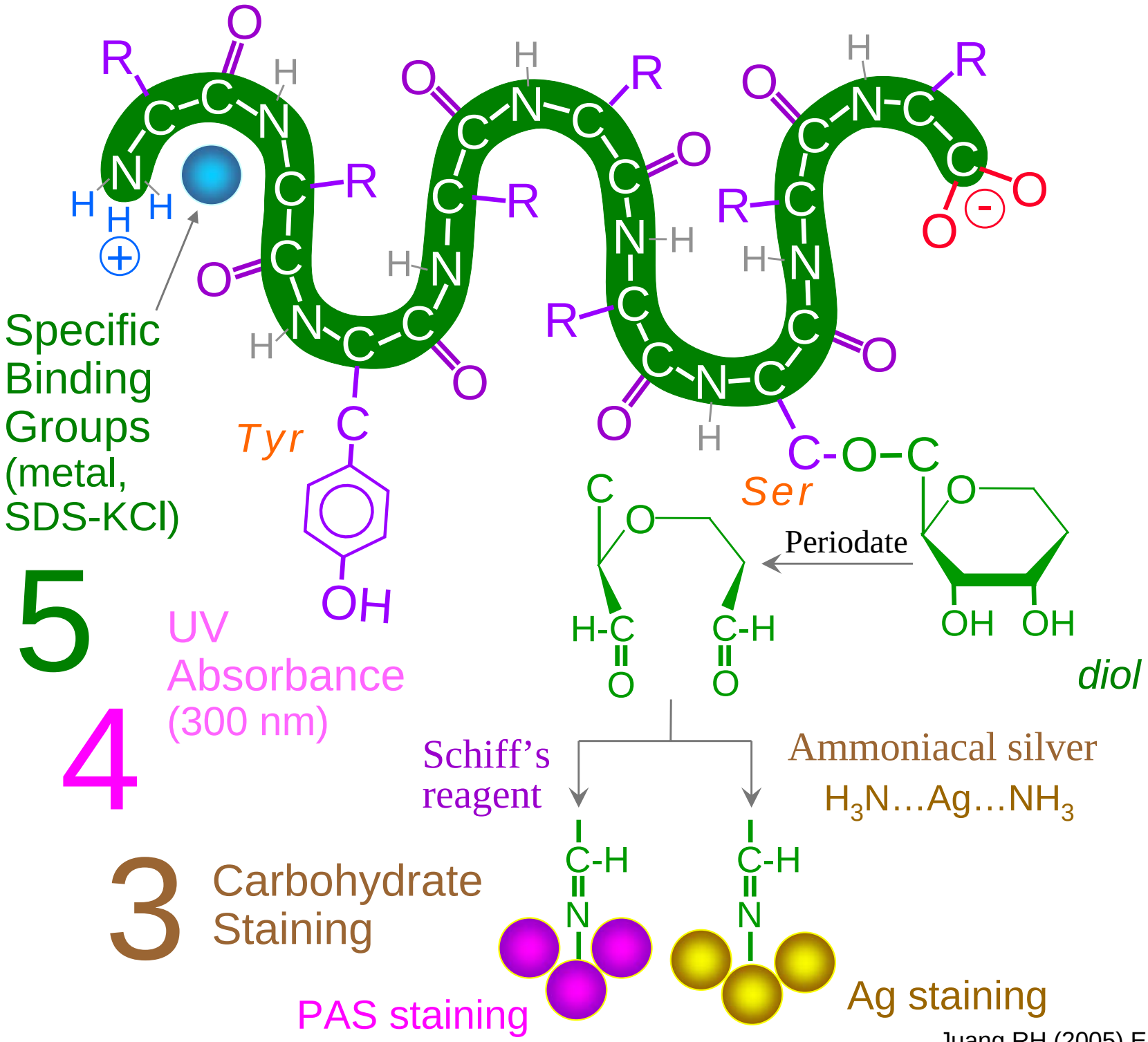
OHC-C-C-C-CHO



Gel staining methods and their mechanism

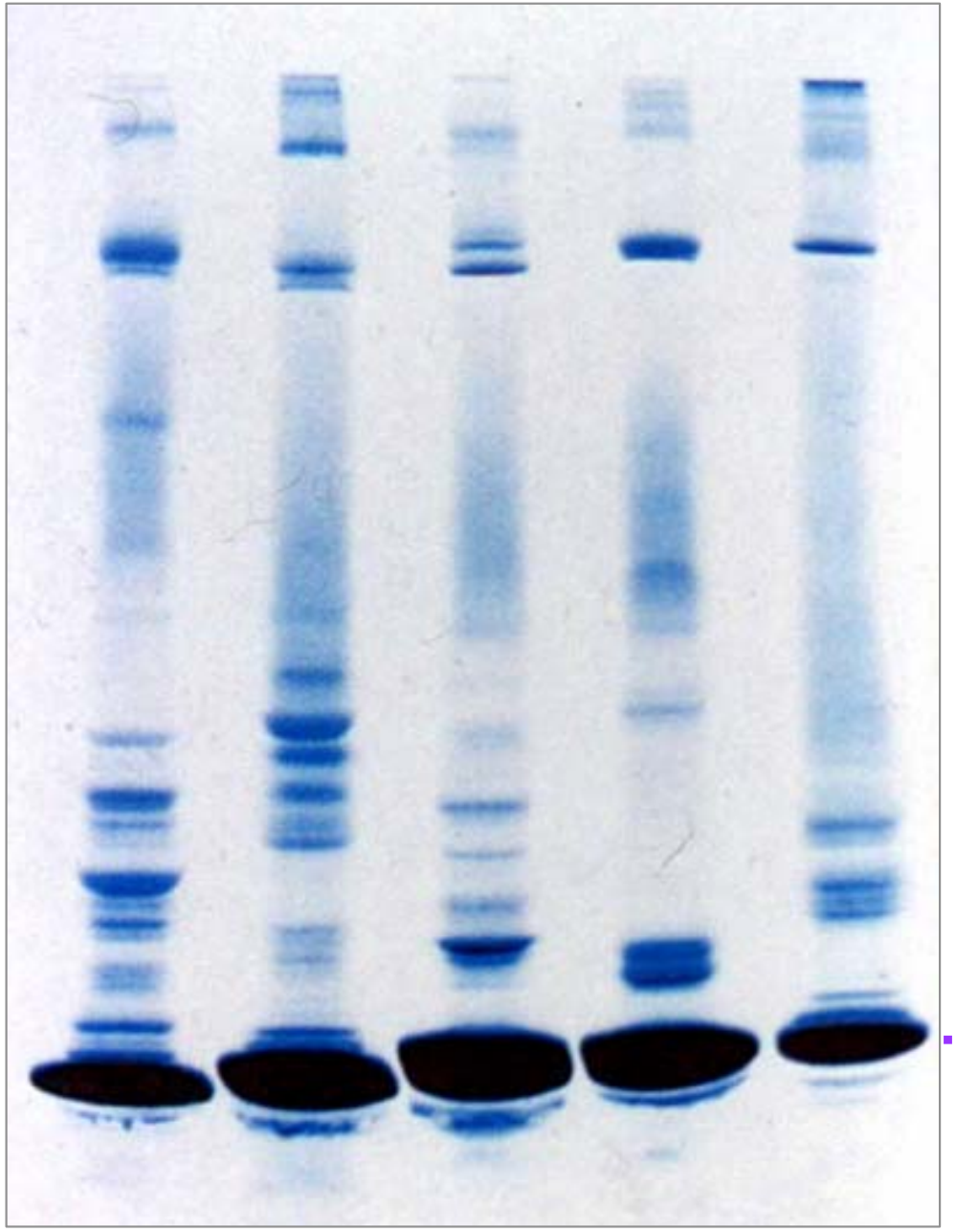
Juang RH (2005) EPA

各種蛋白質染色法機制

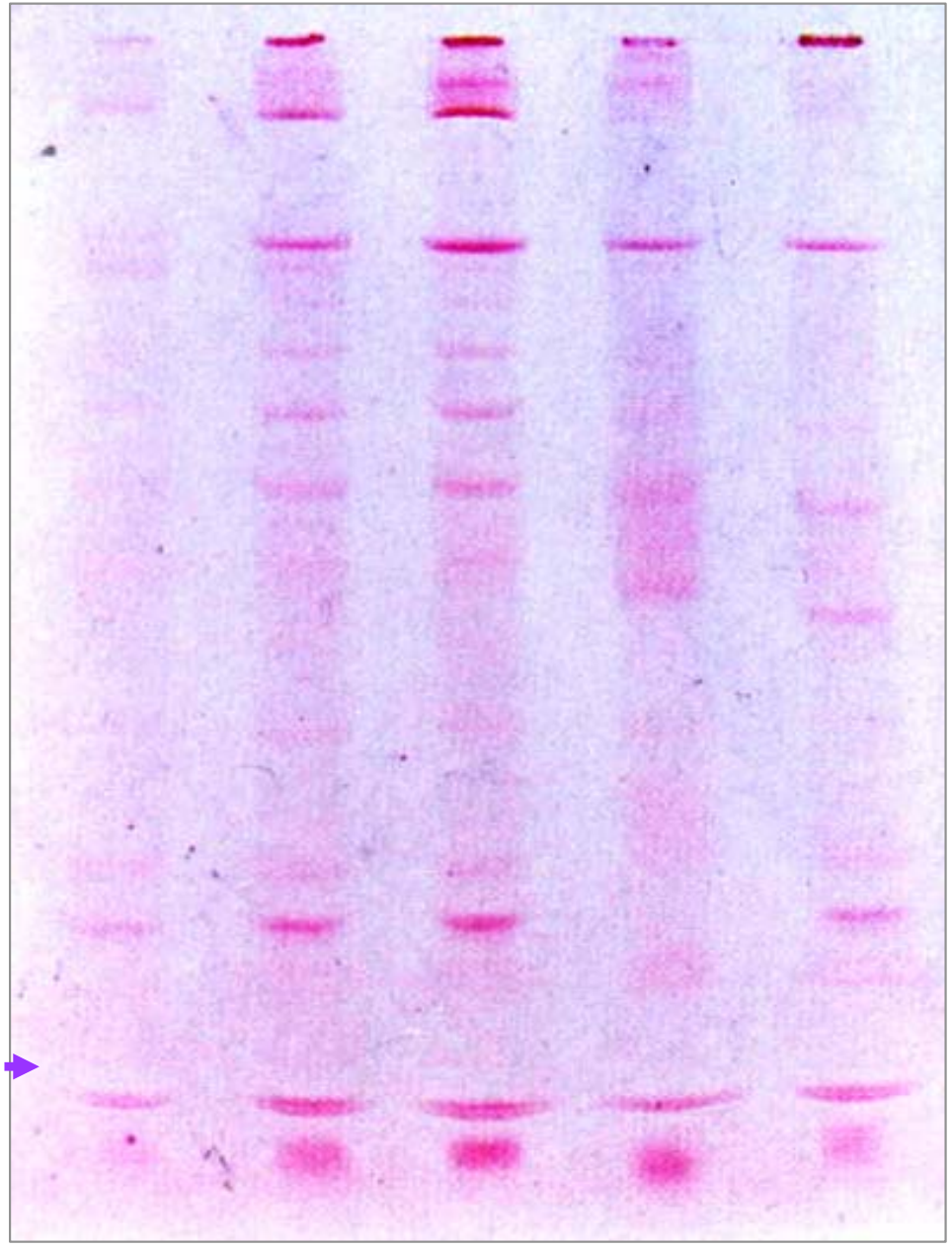


Juang RH (2005) EPA

血清電泳染色結果 Serum stained by CBR / PAS

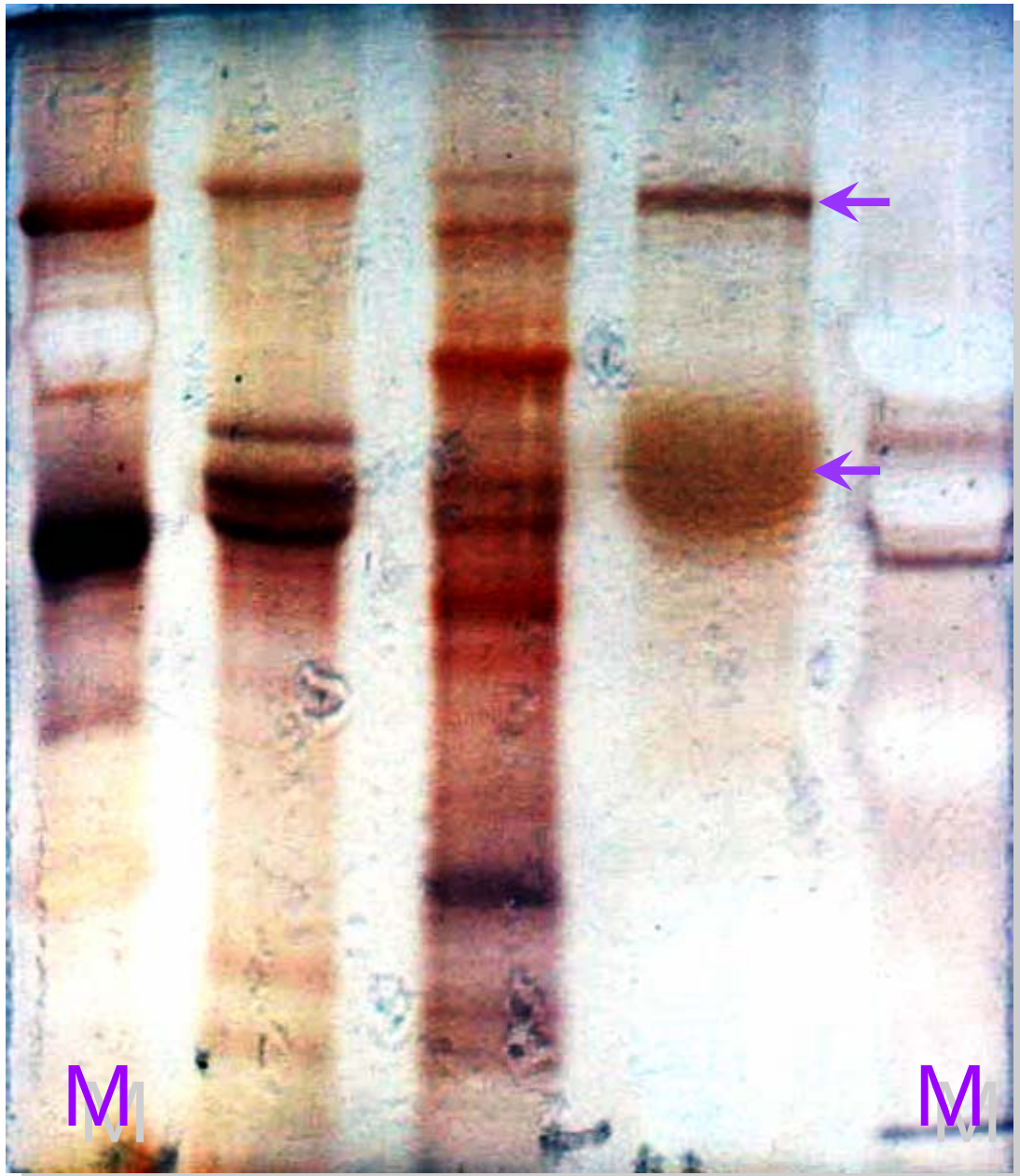


Pharmacia: Electrophoresis



Albumin can't be stained with PAS

SP 醣染色結果 Glycoprotein stained with $AgNO_3$

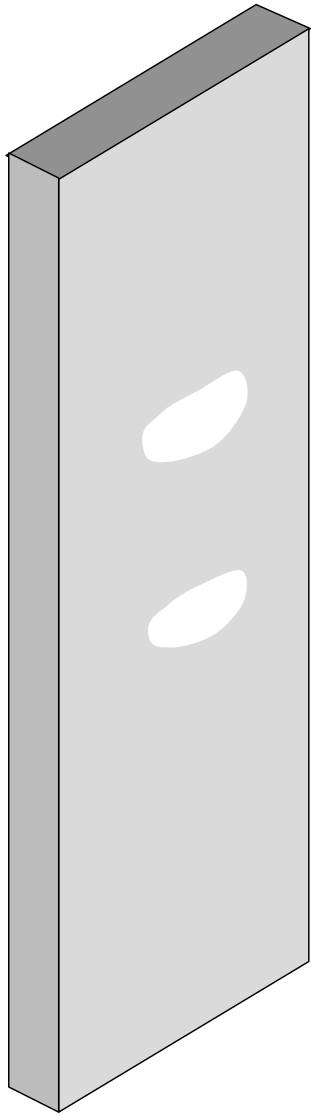


Juang RH (2005) EPA

澱粉磷解酶活性分析及干擾

A

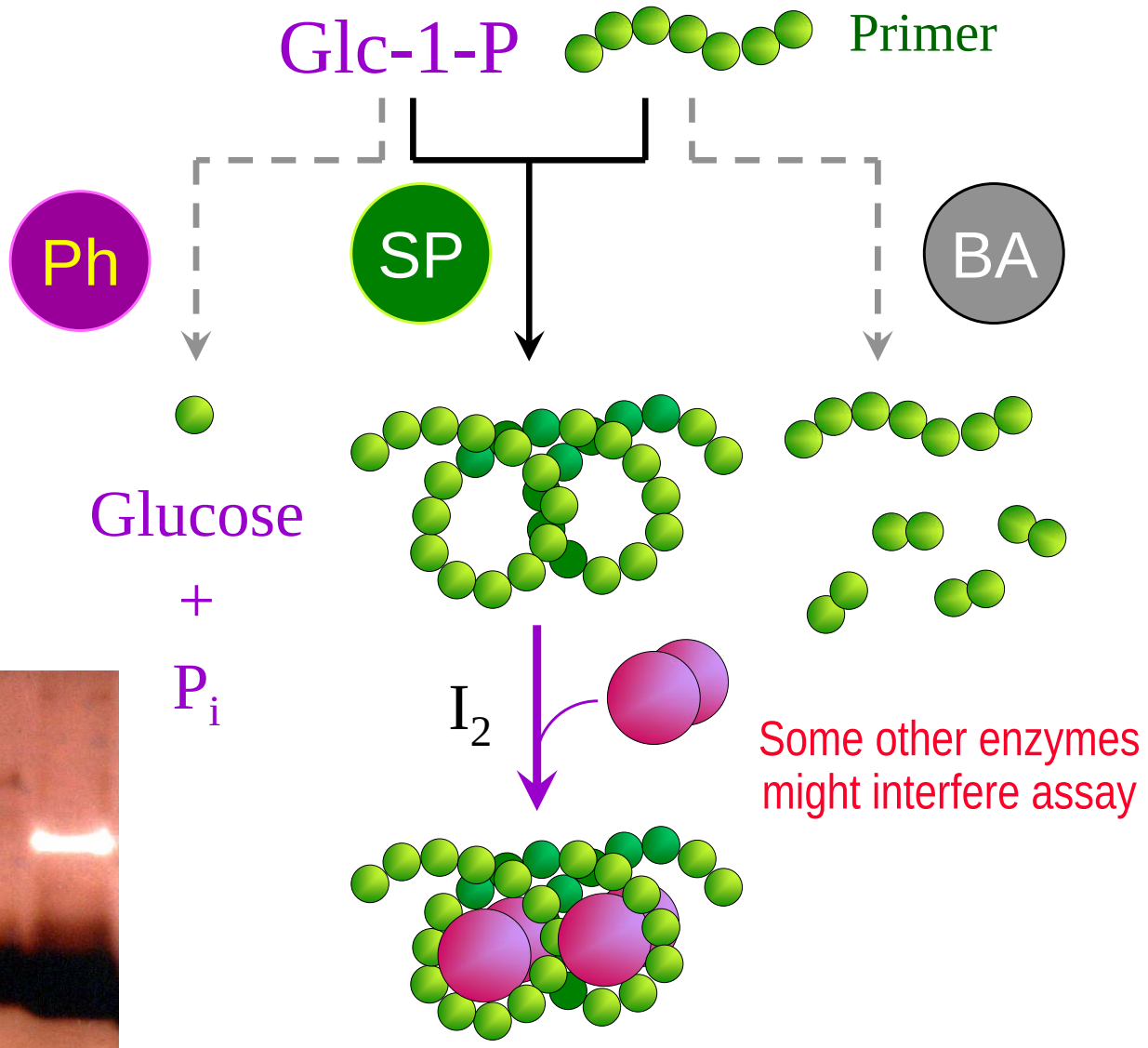
Activity staining



Native PAGE

B

Activity assay and interference



Direct observation of the color product

Not every enzyme can be stained by its activity on the gel

Juang RH (2005) EPA

膠片呈色法比較 Comparison of staining methods

Coomassie Brilliant Blue R-250 Staining	Protein	Med sensitivity ●●	Simple, rapid
Ammoniacal Silver Staining	Protein	High sensitivity ●●●	Complex steps
Periodic Acid - Schiff's Reagent	Carbohydrate	Low sensitivity ●	Complex steps
UV Absorbance (300 nm)	Protein or nucleic acid	Low sensitivity ●	Illuminate gel directly
Autoradiography	Radioactive labeled molecule	High sensitivity ●●●	Radioactive hazard
KCl Precipitation	Protein coated with SDS	Low sensitivity ●	Simple, rapid
Activity Staining	Enzyme reaction (insoluble product)	High sensitivity ●●●	Variable

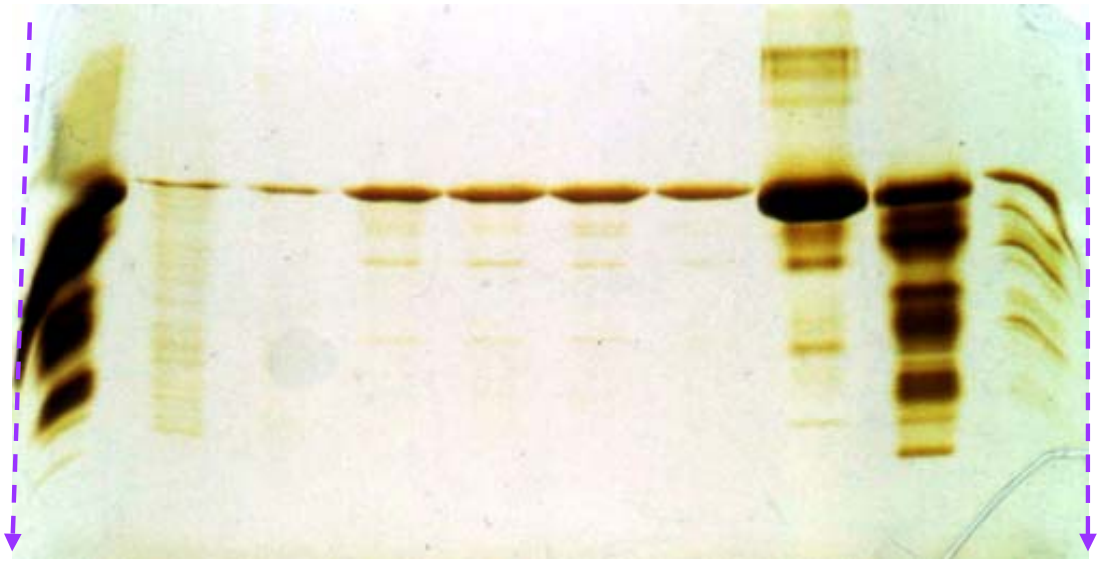
Juang RH (2005) EPA

電泳結果不佳十大原因 Trouble shooting (Top 10)

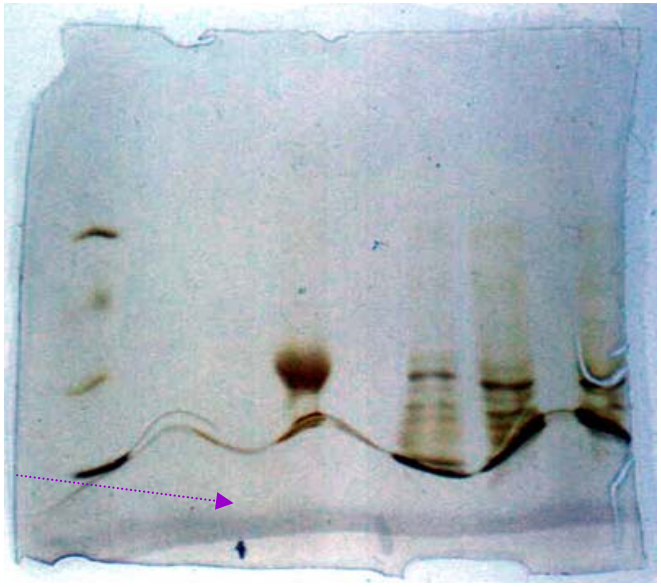
Can't polymerize	Wrong APS concentration or inactivated APS
Band twisting	Bad gel casting or gel trapped bubbles
Band diffusing, tailing	Samples have high-salt or extreme pH
Some lanes failed	Sample wells cleaned (tooth-brushing) ?
Partly polymerized	APS not dissolved completely?
Dye front line leaned	Uneven temperature or leaking electrode buffer
Gel with vertical lines	Impurity in gel or reagents
No stacking effect	Check *p*H of C solution (stacking gel)
Wrong mobility	Forgot SDS in gel, buffer or sample?
Gel became sticky	Forgot Bis in gel solution?

Juang RH (2005) EPA

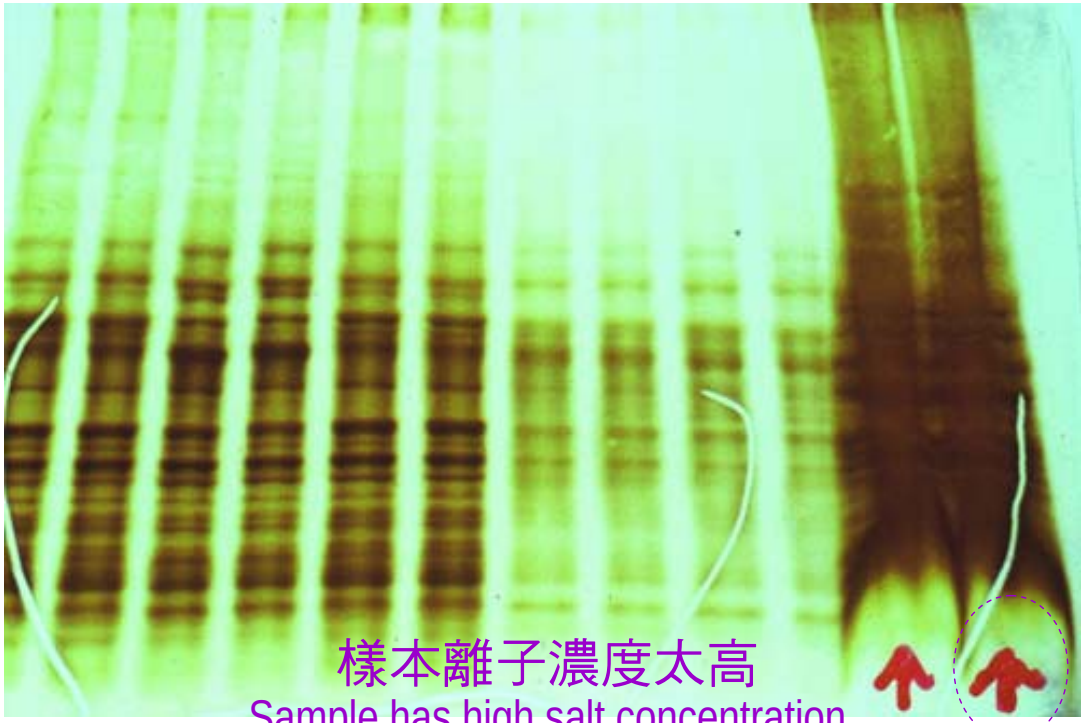
各種電泳染色的問題 Some gel problems



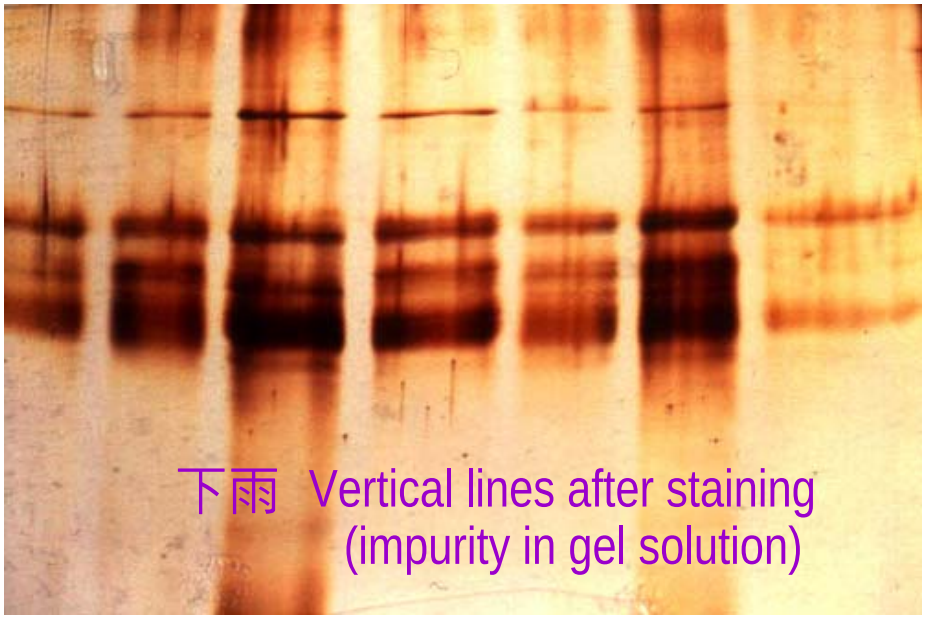
暗流
Leaking buffer conducting the electric current



凝膠不均勻
Gel is unevenly polymerized



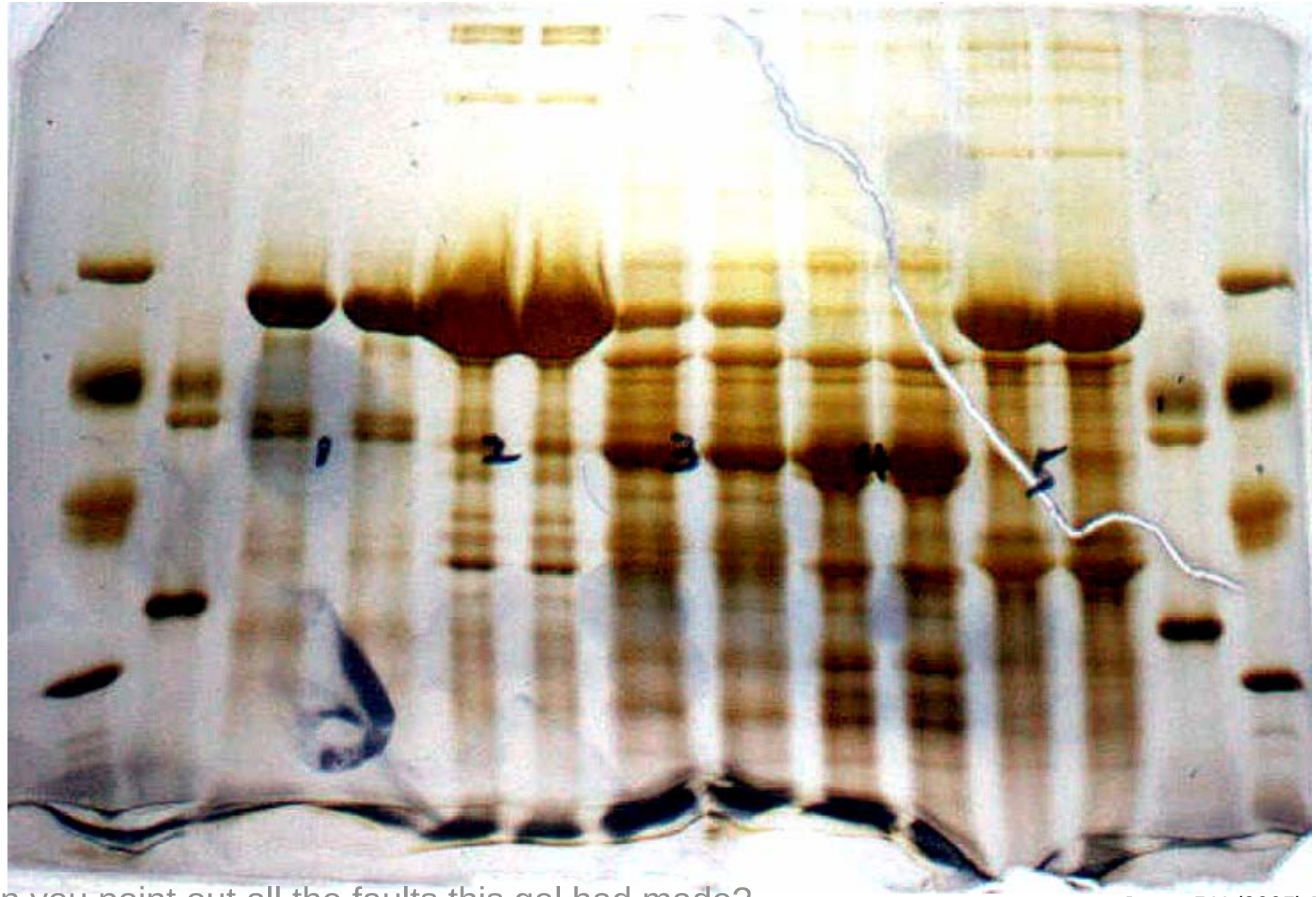
樣本離子濃度太高
Sample has high salt concentration



下雨 Vertical lines after staining (impurity in gel solution)

Juang RH (2005) EPA

各種電泳染色的問題 A malformed gel



Can you point out all the faults this gel had made?

Juang RH (2005) EPA

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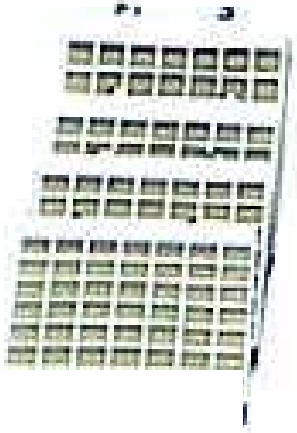
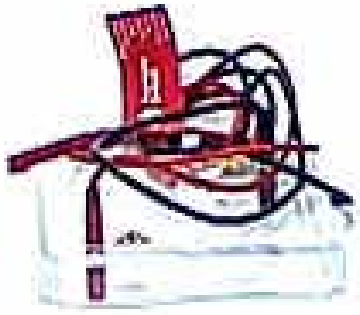
電泳槽及相關設備 Instruments and equipments

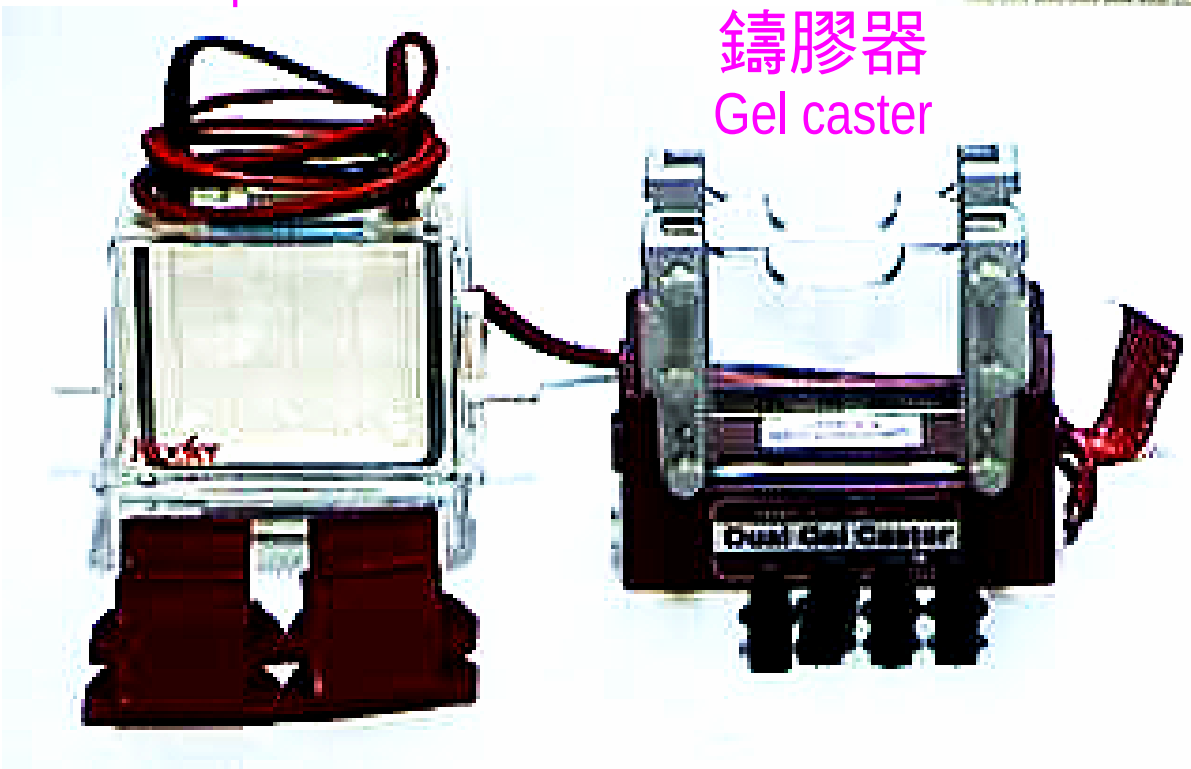
轉印三明治
Transfer sandwich



轉印槽
Gel transfer



電泳槽
Electrophoresis unit



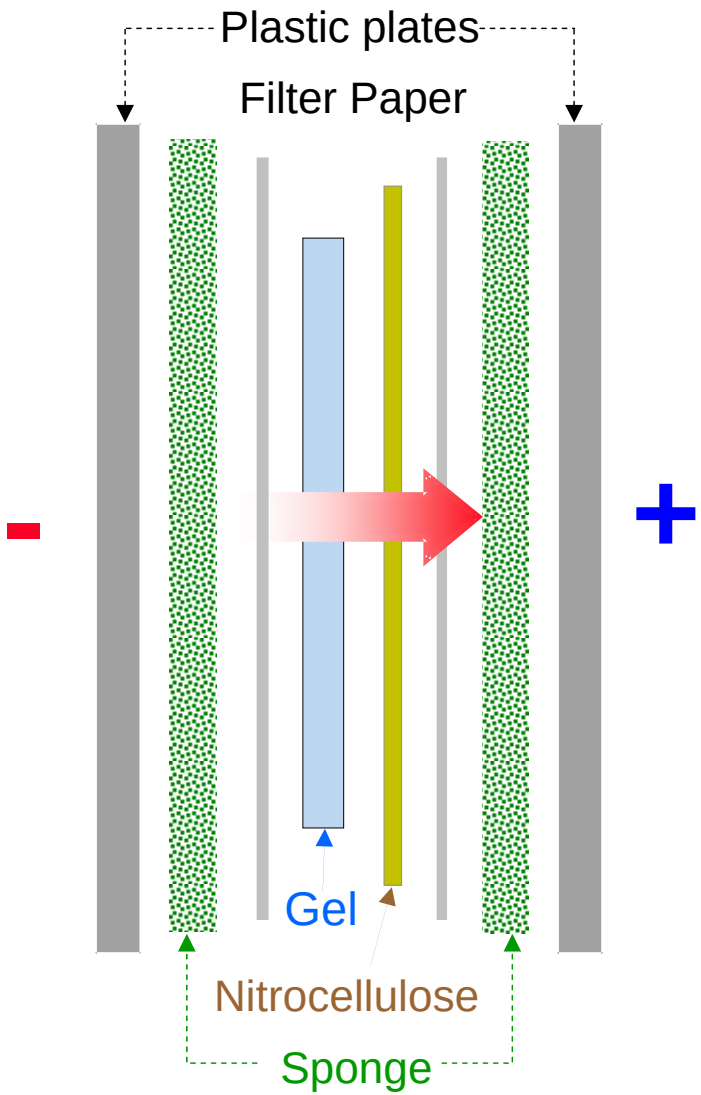
鑄膠器
Gel caster

供電器 Power supply

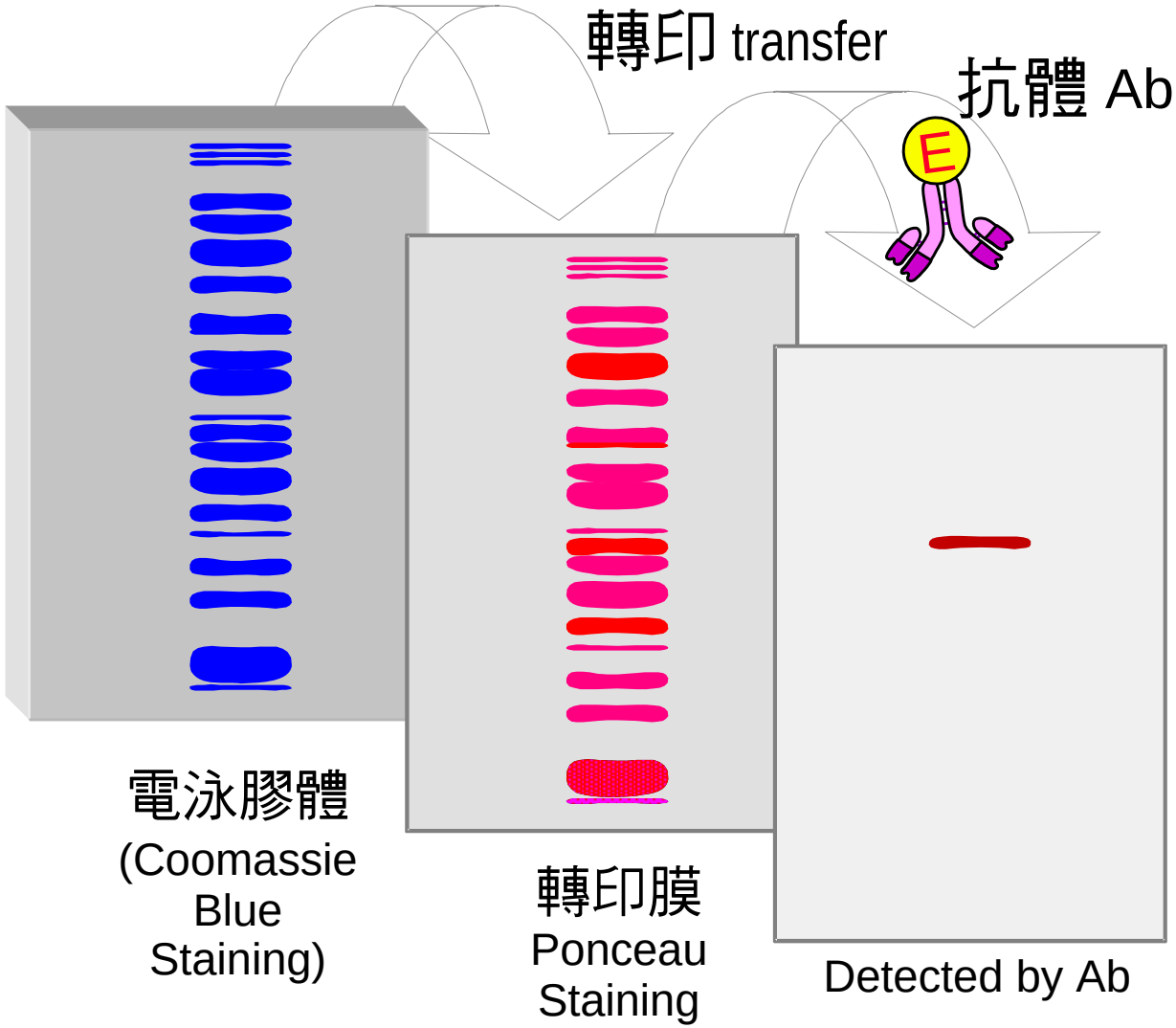
Juang RH (2005) EPA

轉印及免疫染色 Protein transfer and staining

A 轉印三明治
Transfer sandwich

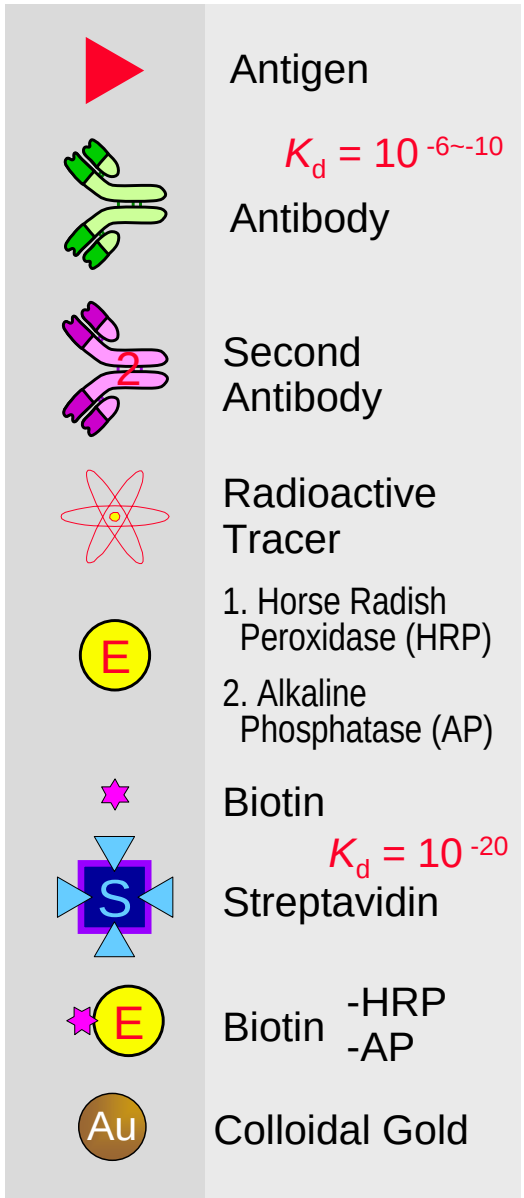


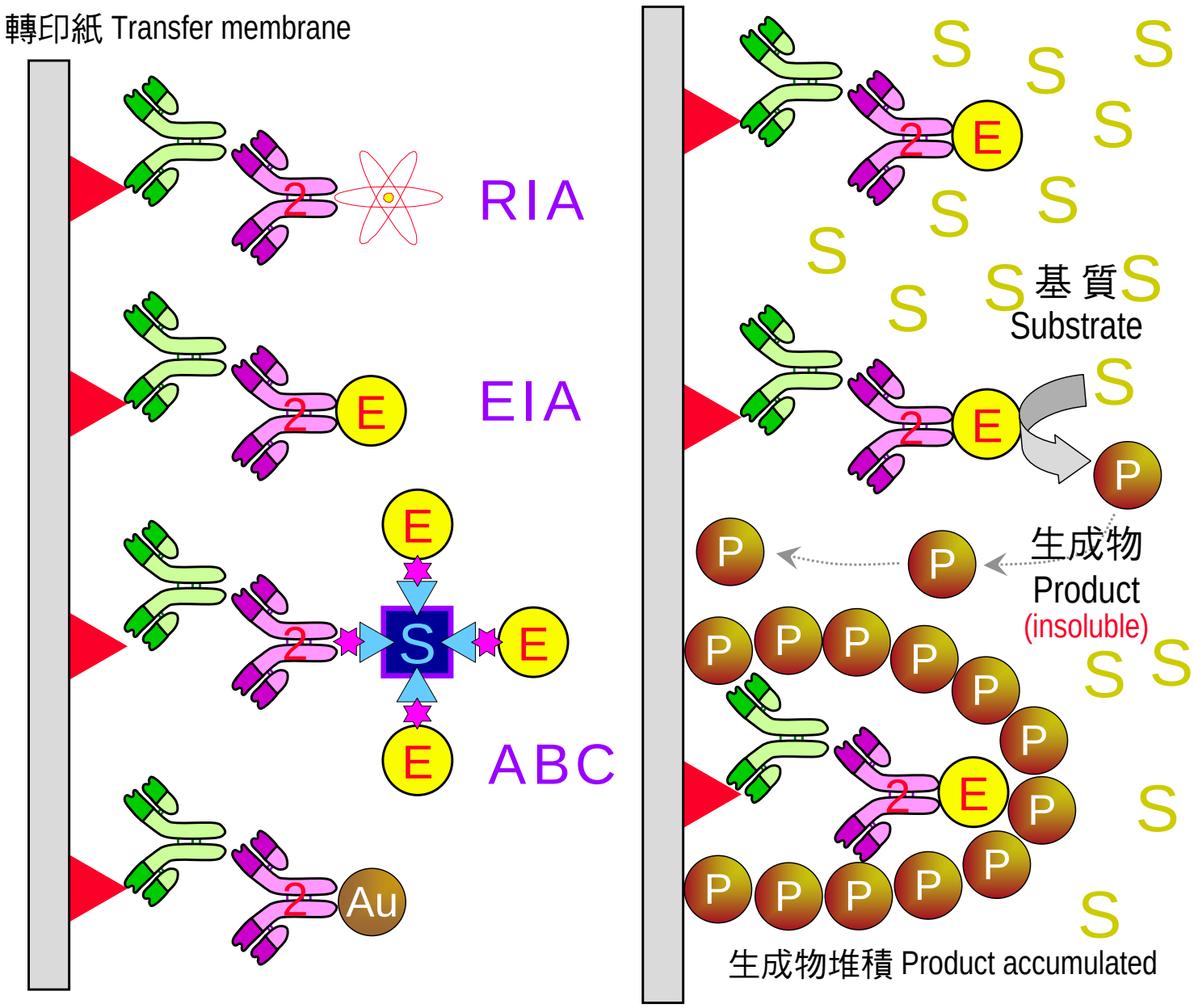
B 免疫染色流程及結果
Immunostaining procedure and result



Juang RH (2005) EPA

免疫轉印種類與呈色機制 Immunoassays



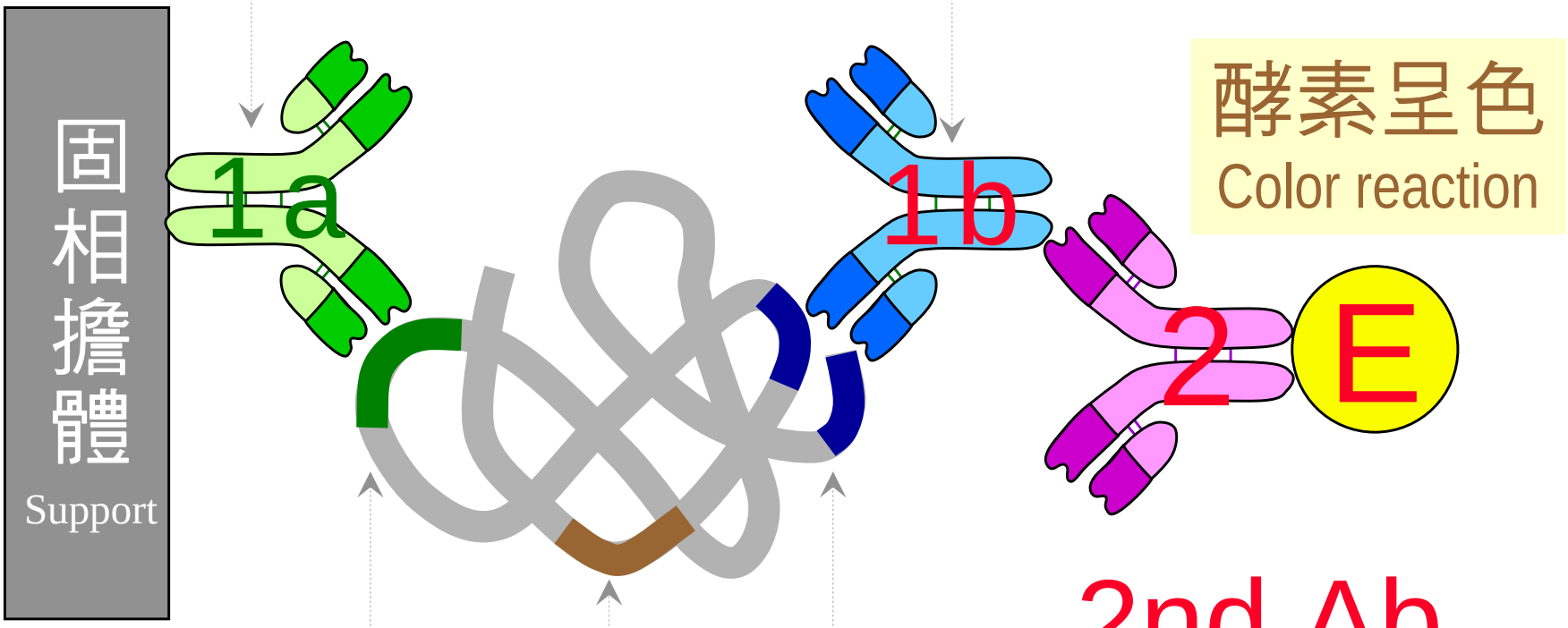


Juang RH (2005) EPA

三明治免疫分析法 Sandwich ELISA method

使用不同動物來源的兩種抗體

Use two Ab from different animal sources



抗原要有多個抗原決定基

Ag contains at least two epitopes

Juang RH (2005) EPA

標誌酵素及呈色劑

Peroxidase

Horse radish peroxidase (HRP) 山葵過氧化酶

Substrate: DAB (brown) 4CN (blue)

Sensitivity: 500 pg

Phosphatase

Alkaline phosphatase (AP) 鹼性磷酸酶

Substrate: BCIP + NBT (blue)

Sensitivity: 100 pg

Substrate: AMPPD (Chemiluminescenct)

Sensitivity: 10 pg (very sensitive)

Enzymes used in 2nd Ab-enzyme conjugates

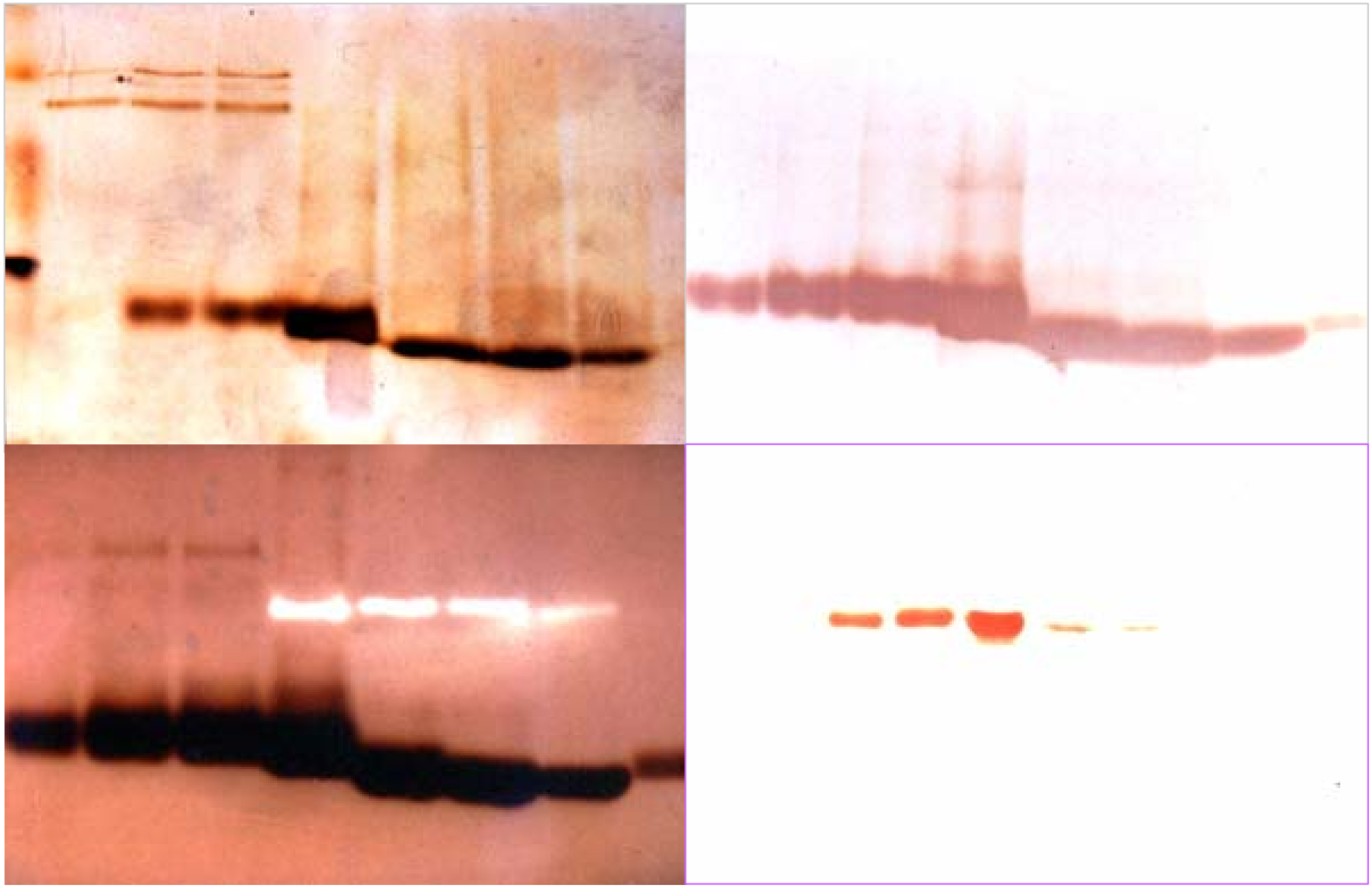
澱粉磷解酶染色方法比較 Staining L-SP

Gel filtration fractions

Immunostaining

Silver staining

Activity staining



Disc-PAGE

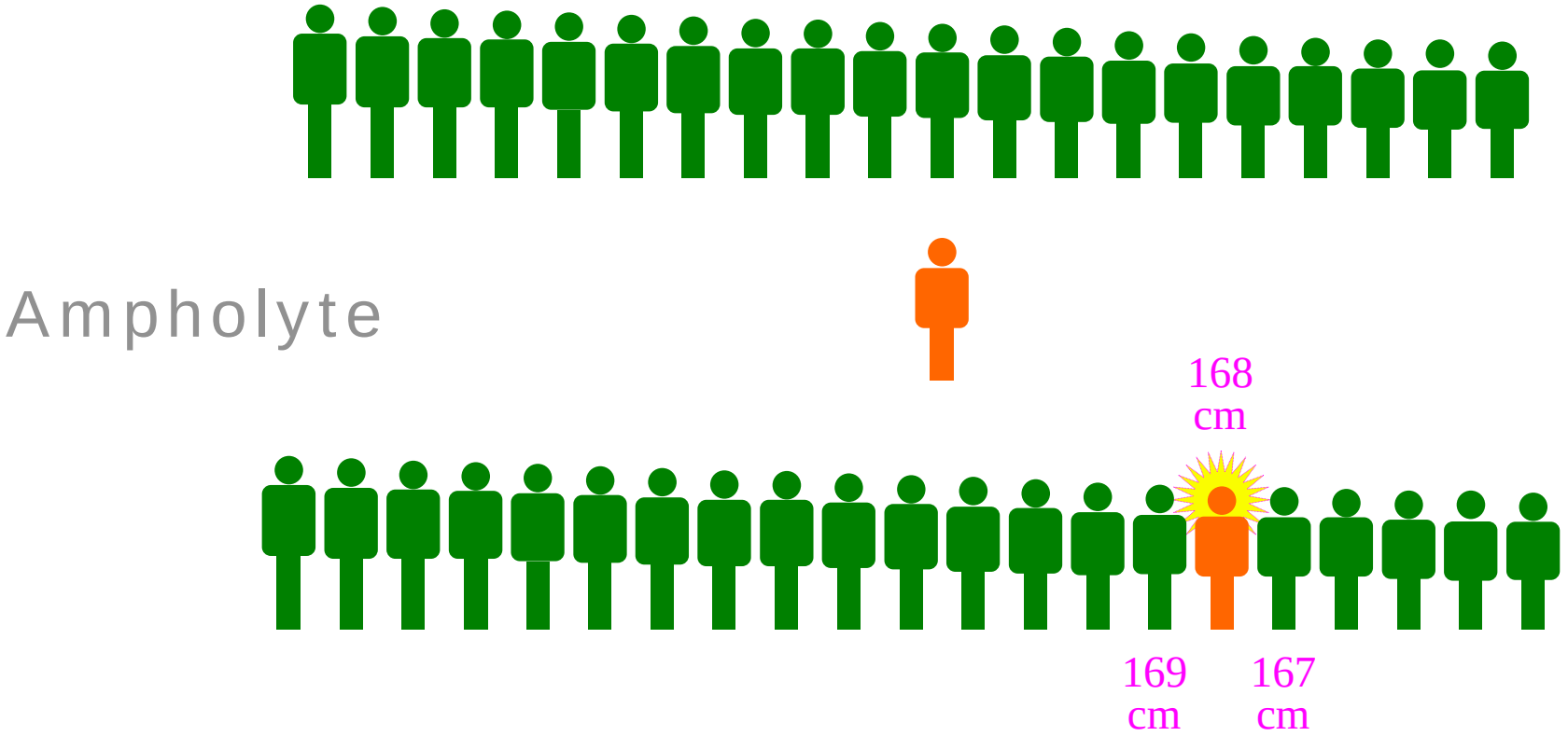
SDS-PAGE

Juang RH (2005) EPA

3.3.2 等電焦集原理 Principle for isoelectric focusing

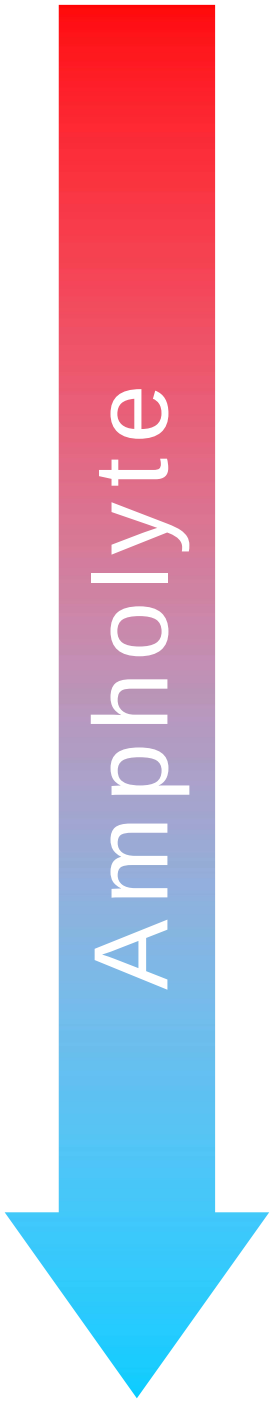
樣本分子 在一已知 pH 梯度 中焦集

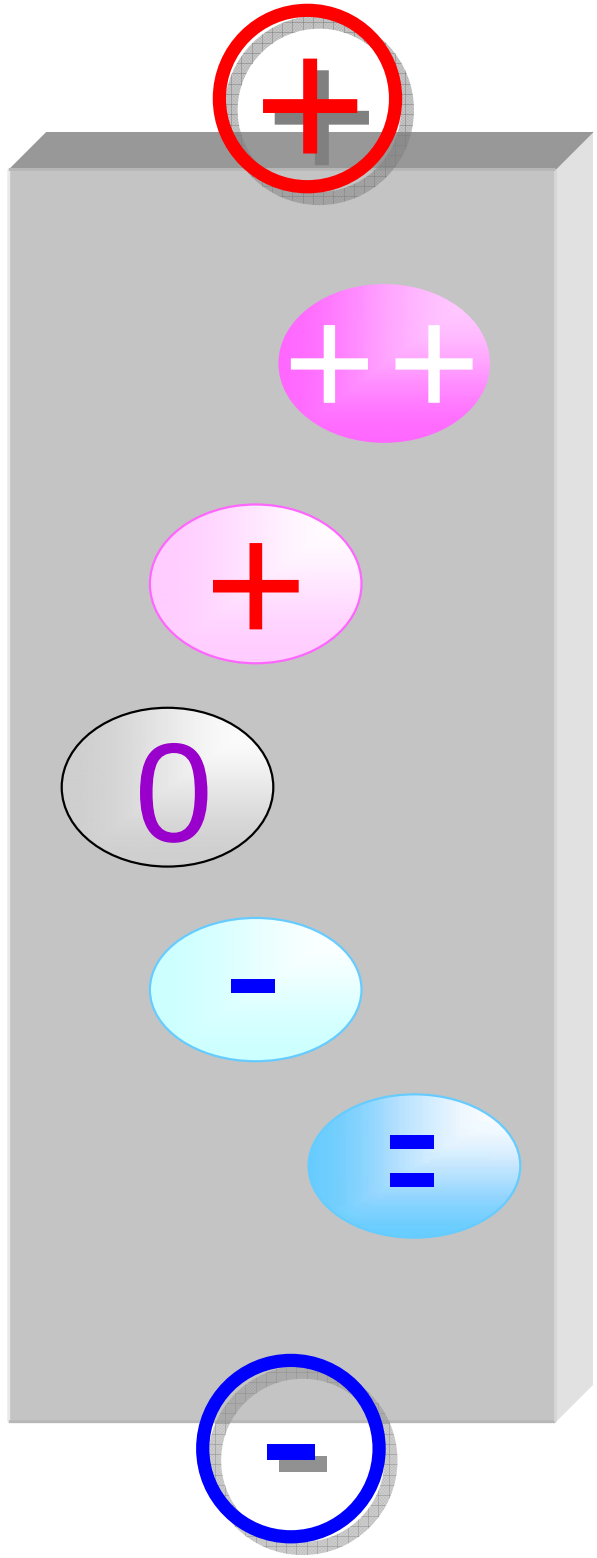
Sample molecules are focused in a preformed pH gradient



Juang RH (2005) EPA

等電焦集法的焦集方式





在低 pH 處帶正電被排斥

At lower pH gel, sample is positively charged and repelled

焦集在等電點

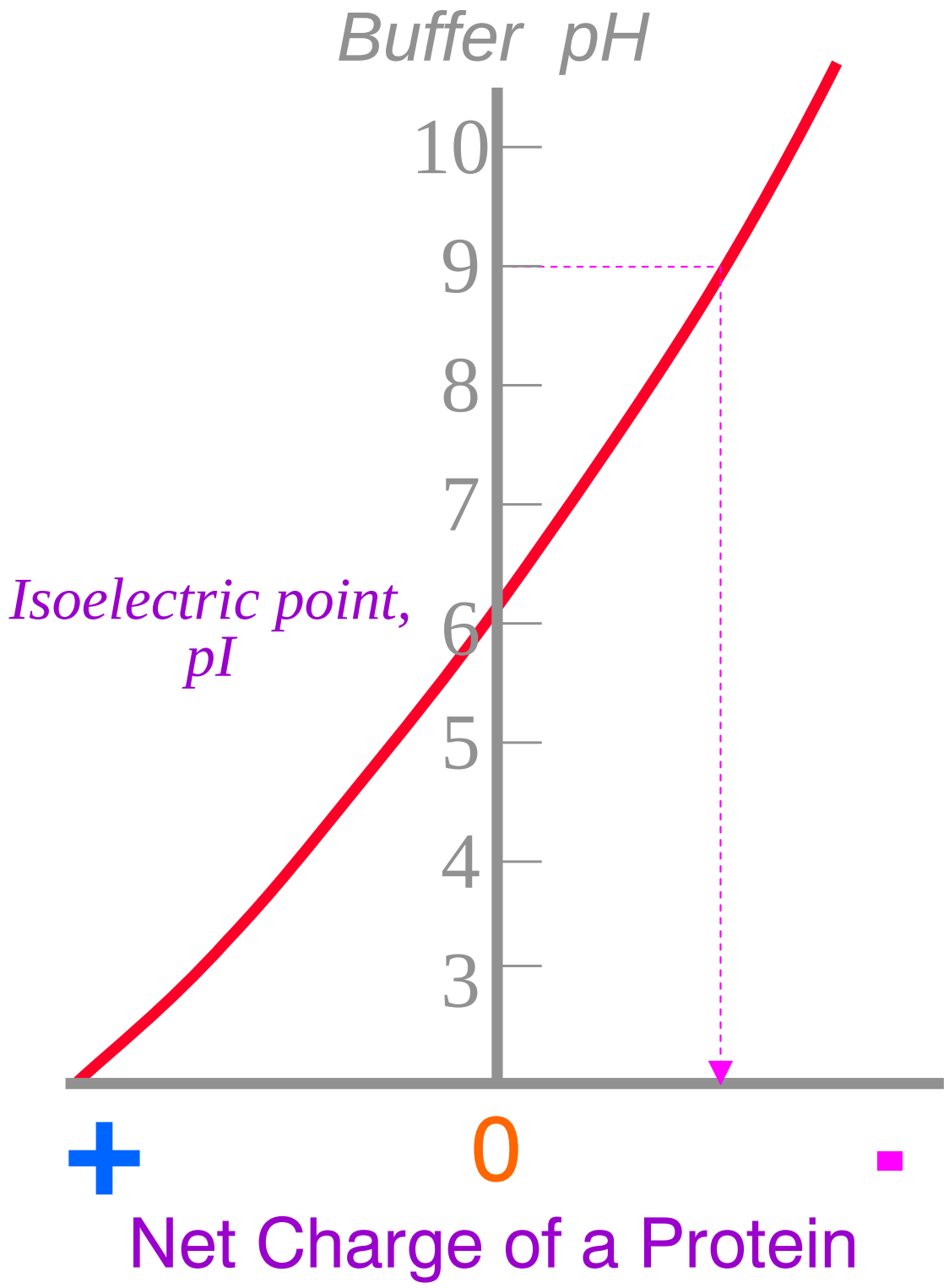
Focused at its pI

在高 pH 處帶負電亦被排斥

At higher pH, sample is negatively charged and also repelled to move upward

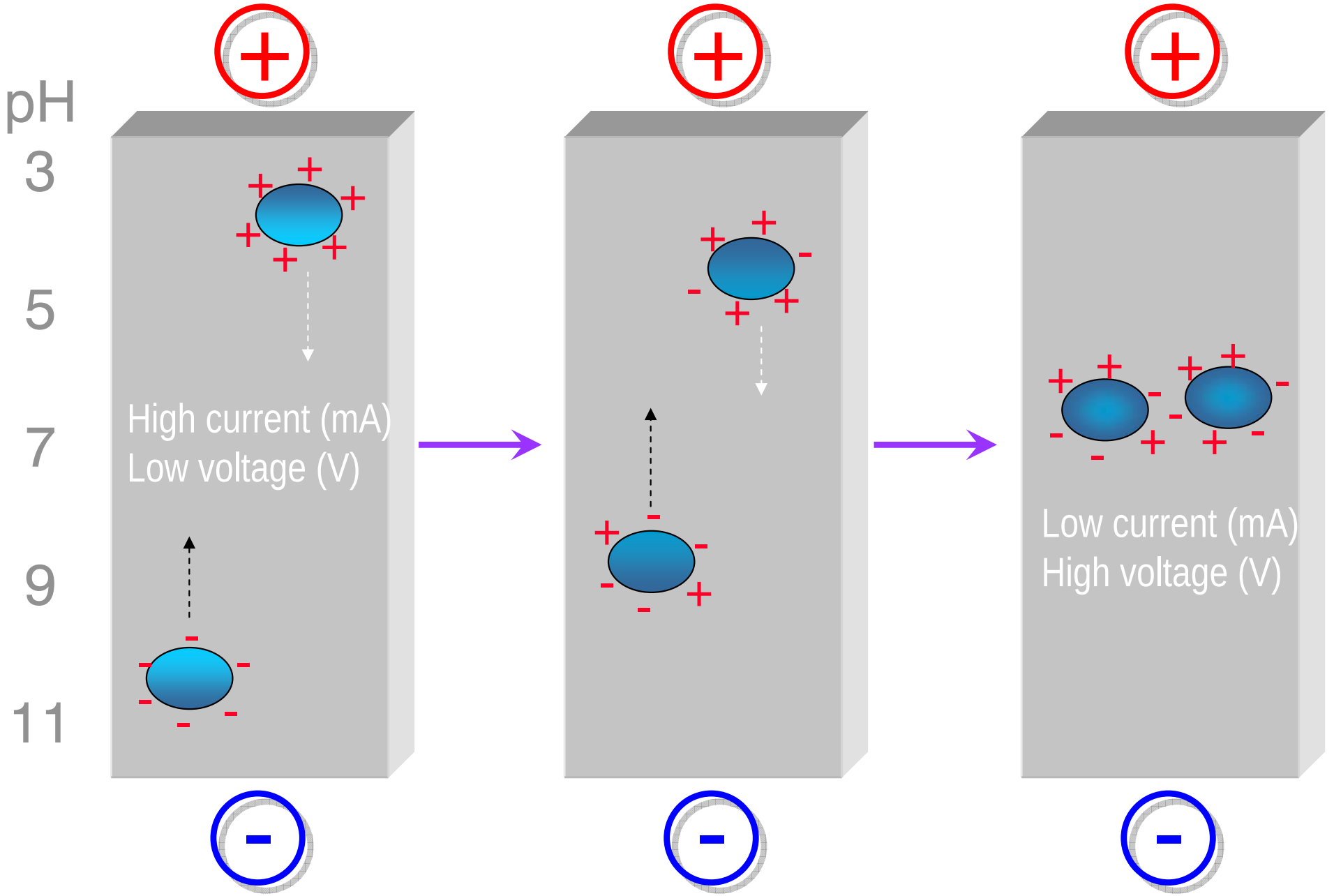
Juang RH (2005) EPA

環境影響分子的帶電性質



Juang RH (2005) EPA

等電焦集的運作機制 Action mechanism of IEF



Adapted from Alberts et al (2002) Molecular Biology of the Cell (4e) p.487

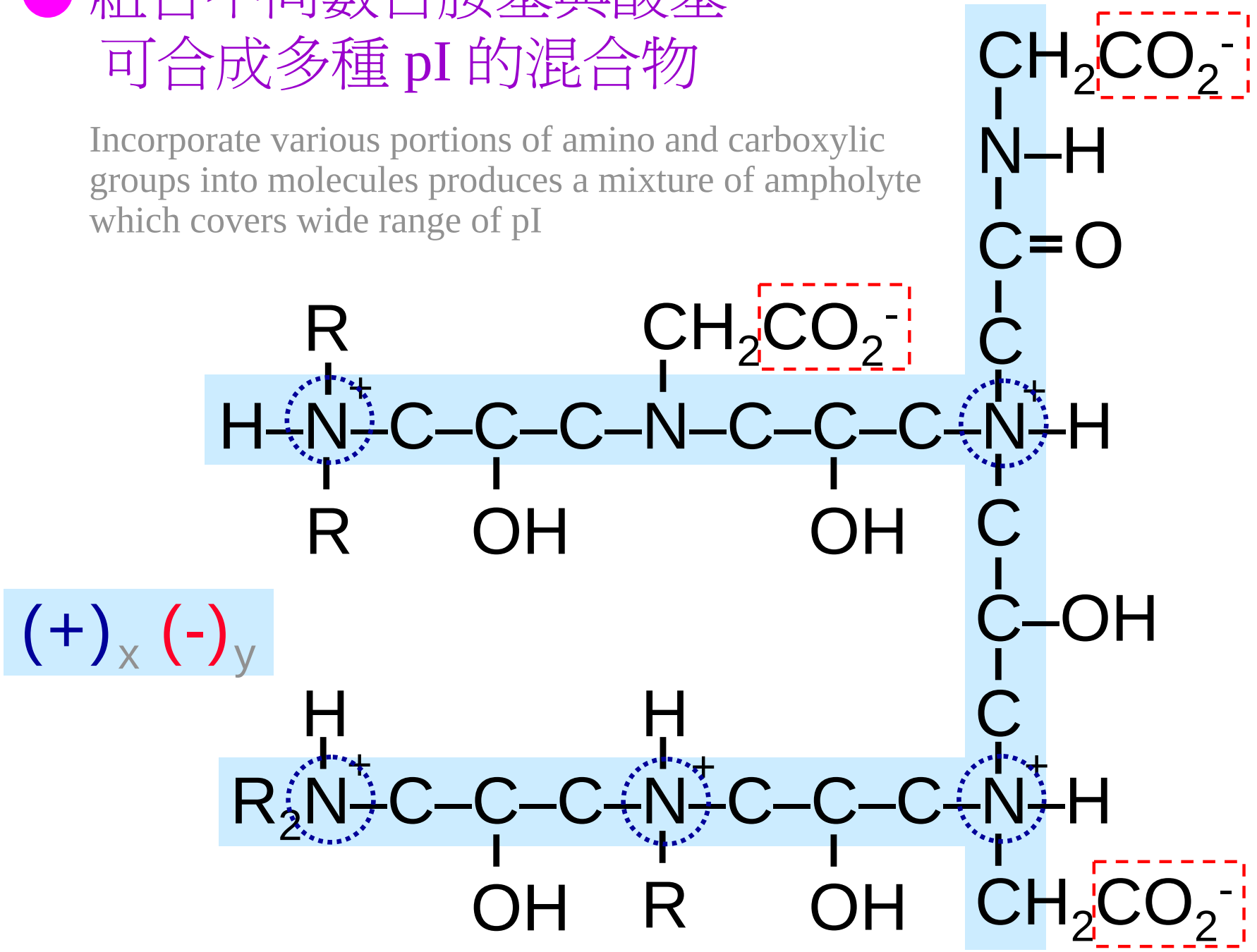
Sample can be applied at any place on the gel

Juang RH (2005) EPA

等電焦集法 雙性離子構造

● 組合不同數目胺基與酸基
可合成多種 pI 的混合物

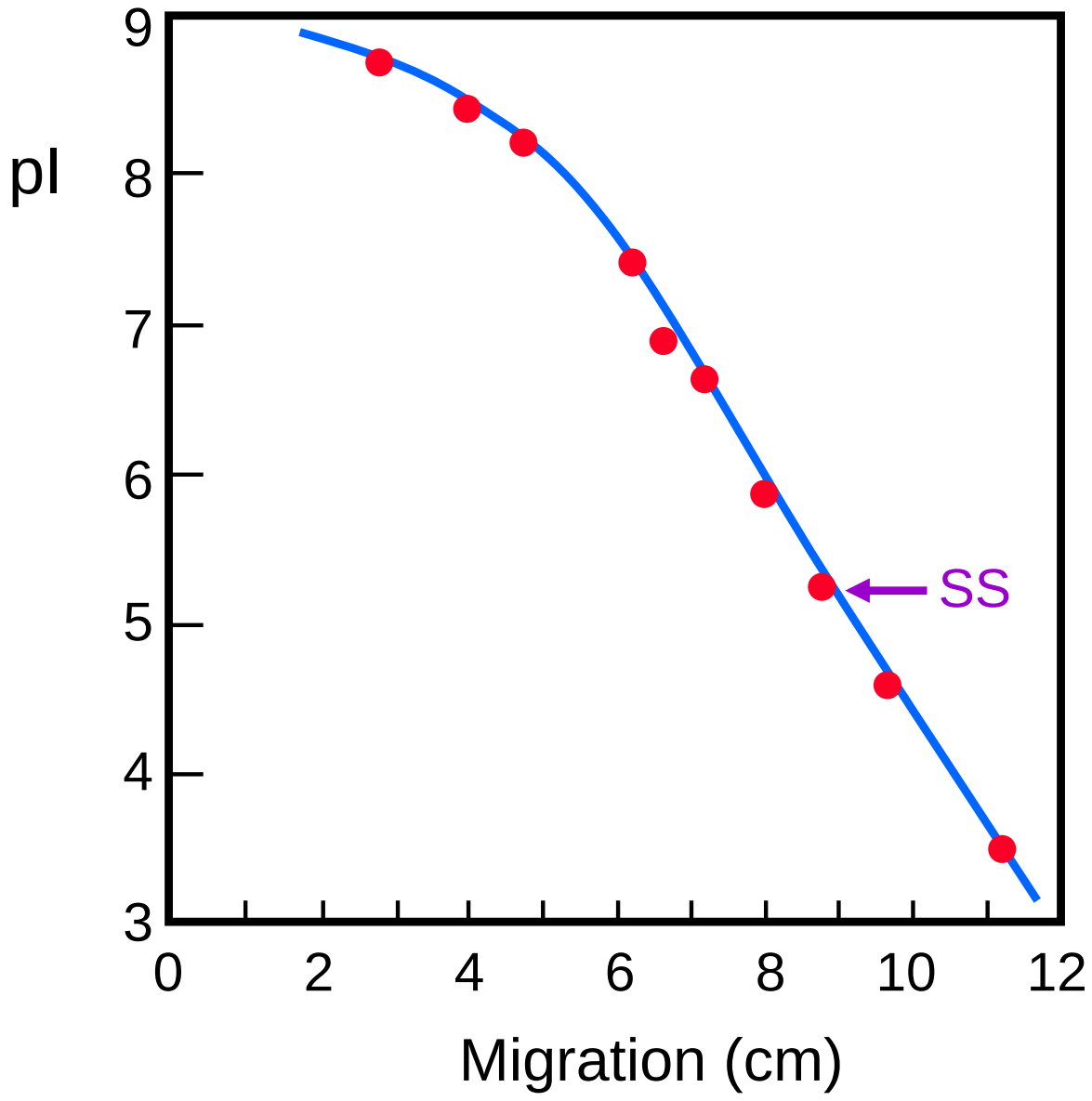
Incorporate various portions of amino and carboxylic groups into molecules produces a mixture of ampholyte which covers wide range of pI



The mixture of ampholytes are synthesized by combinatory chemistry

Juang RH (2005) EPA

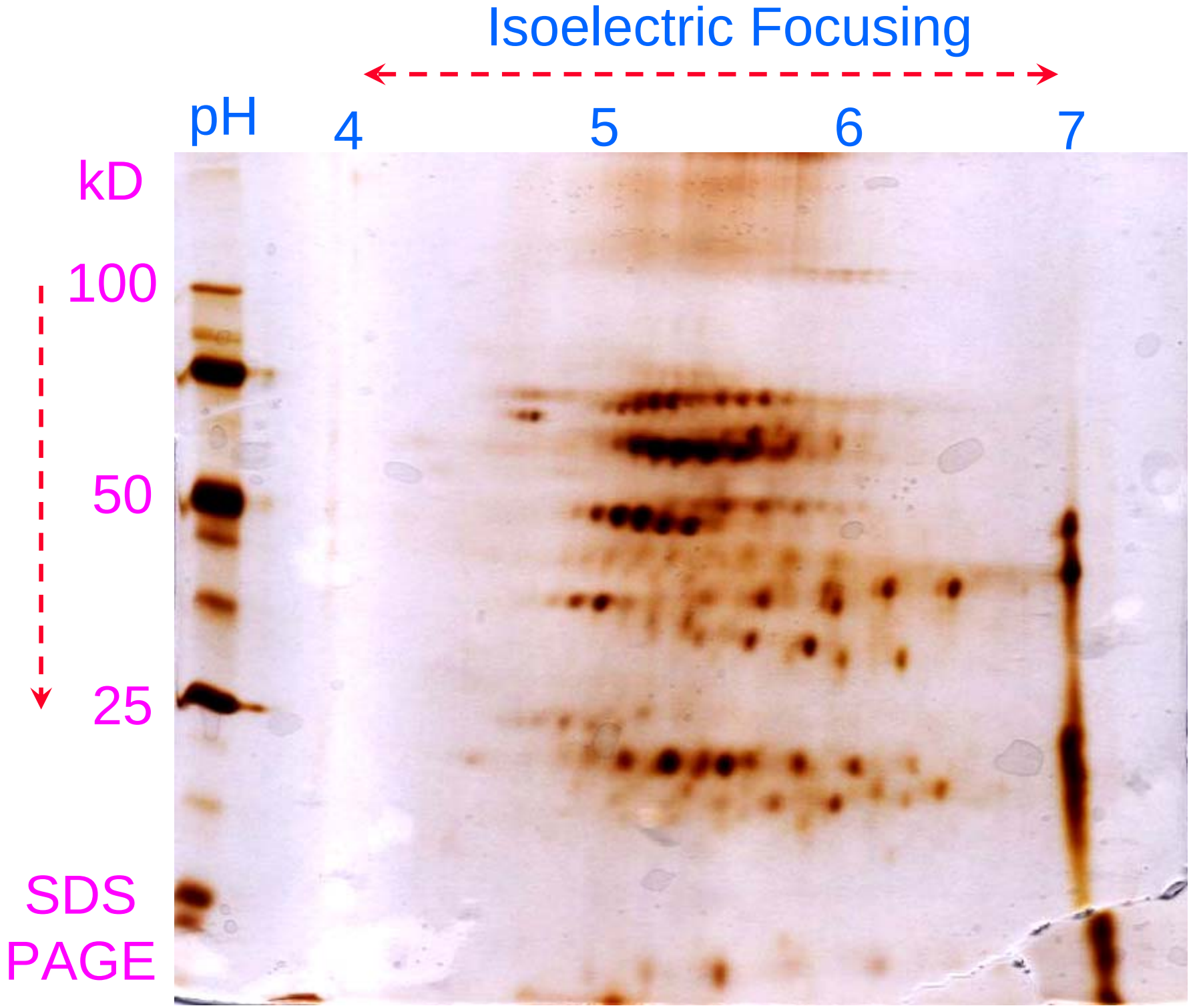
等電點標準校正線 Standard curve for IEF



Standard curve is established by proteins with known pI

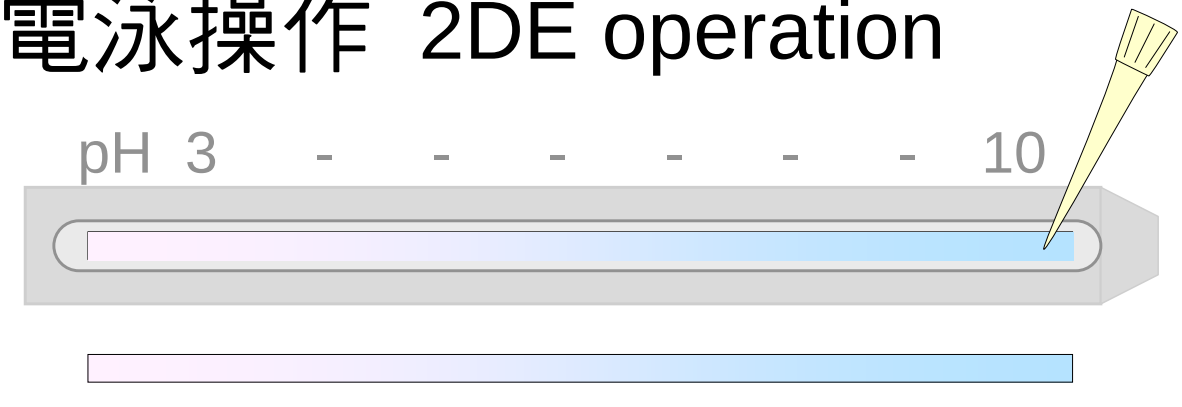
Juang RH (2005) EPA

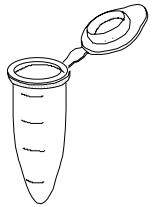
二次元電泳

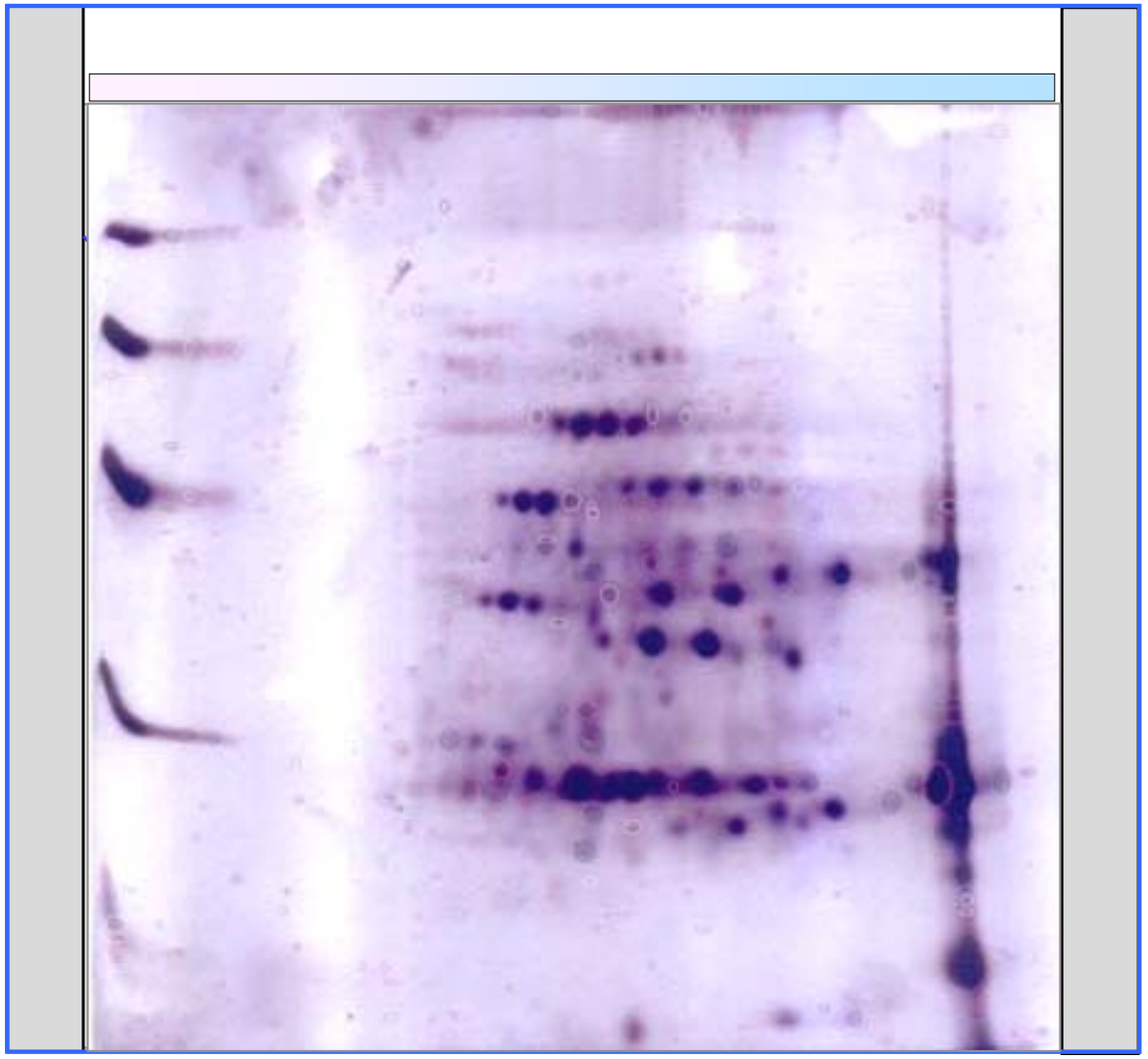

Isoelectric Focusing
pH
4
5
6
7
kD
100
50
25
SDS
PAGE
Juang RH (2005) EPA

二次元電泳操作 2DE operation

(1) IEF
等電焦集電泳







(2)
SDS-PAGE
分離膠體

(3)
Staining
染色脫色

Juang RH (2005) EPA